ABSTRACT

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Title of diploma thesis: DNA-protein covalent complexes detection as the means for the assessment of the DNA damage induced by topoisomerase poisons.

Topoisomerase II is essential cellular enzyme, which modifies the secondary structure of DNA. By introducing a temporary double strand break to DNA it relieves a structural tension raised during transcription and translation. Absolutely indispensable is the role of topoisomerase II in the separation of sister chromatids synthesized in the S-phase of the cell cycle. The mechanism of DNA cleavage involves a covalent bond formed between active site tyrosine and 5‘ phosphate on both of the DNA strands and through the formed break the other strand or the other DNA molecule can pass. After that, the DNA strands are rejoined and topoisomerase II is detached.

The indispensability of topoisomerase II mainly for proliferating cells makes it a great target for the antineoplastic drugs and the molecules belonging to the class of topoisomerase II inhibitors (etoposide, anthracyclines) are amongst the most useful anticancer drugs in the clinical practice. These clinically used „topoisomerase poisons“ act by stabilizing the covalent complex of topoisomerase II and the DNA and block the rejoining of the transient DNA break, which stops the cell cycle and can lead to permanent double strand breaks and DNA damage. Besides the intended antiproliferative effect, the DNA damage can also be a mechanism of toxicity of these drugs.

In the Charles University Research Centre for the Study of Toxic and Protective Effects of Drugs on Cardiovascular System (UNCE 204019/304019/2012) the mechanisms of anthracycline cardiotoxicity are studied. Topoisomerase II inhibition is obviously the mechanism of anthracycline antineoplastic action, but recent

investigations of this and other groups suggest, that it could be involved in the mechanisms of cardiotoxicity, which are still elusive. One of the approaches which could clarify this hypothesis could be the assessment of the level of topoisomerase II complexes with DNA formed upon the treatment of cardiomyocytes or cancer cells with anthracyclines and its comparison with the direct DNA damage measured using different method.

The aim of this thesis was to optimize the method of detection of the topoisomerase II covalent complexes with DNA first in the HeLa cell line induced by etoposide and the comparison with direct assessment of the DNA damage by the Comet Assay. This optimization will enable further studies on different cell lines or the primary culture of isolated rat neonatal cardiomyocytes and using different topoisomerase II poisons (*e.g*. anthracyclines).